Translational research to improve the treatment of severe extremity injuries

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ABSTRACT

Objectives Severe extremity injuries are the most significant injury sustained in combat wounds. Despite optimal clinical management, non-union and infection remain common complications. In a concerted effort to dovetail research efforts, there has been a collaboration between the UK and USA, with British military surgeons conducting translational studies under the auspices of the US Institute of Surgical Research. This paper describes 3 years of work.

Methods A variety of studies were conducted using, and developing, a previously validated rat femur critical-sized defect model. Timing of surgical debridement and irrigation, different types of irrigants and different means of delivery of antibiotic and growth factors for infection control and to promote bone healing were investigated.

Results Early debridement and irrigation were independently shown to reduce infection. Normal saline was the most optimal irrigant, superior to disinfectant solutions. A biodegradable gel demonstrated superior antibiotic delivery capabilities than standard polymethylmethacrylate beads. A polyurethane scaffold was shown to have the ability to deliver both antibiotics and growth factors.

Discussion The importance of early transit times to Role 3 capabilities for definitive surgical care has been underlined. Novel and superior methods of antibiotic and growth factor delivery, compared with current clinical standards of care, have been shown. There is the potential for translation to clinical studies to promote infection control and bone healing in these devastating injuries.

INTRODUCTION

Extremities remain the most common site of combat injuries.[1 2] Between a third and a half of these injuries are associated with fractures, the majority of which are open fractures.[2 3] These high-energy injuries are associated with significant damage to soft tissue and to bone.[3 4]

Two of the sequelae of saving severely injured limbs are fracture non-union and infection, which both contribute to delayed amputations, that is, an amputation at a later date due to complications from the original injury. Non-union complicates many bone defects and has an incidence of up to 32%, even in civilian severe lower extremity injuries.[5 6] Infection significantly compromises fracture healing[7 8] and is the most common cause of delayed amputation in combat-related open tibial fractures.[9] A review of British military open fractures demonstrated a 40% infection rate.[10] The most commonly isolated pathogen is *Staphylococcus Aureus*,[11] which is also the most frequent isolate in recurrent, recalcitrant osteomyelitis.[12 13]

Current management

Traditional methods for treating non-union, which involve taking a bone graft from elsewhere in the same patient, have significant drawbacks such as donor-site morbidity. More recently, bone has been successfully regenerated using growth factors such as recombinant human bone morphogenetic proteins (rhBMPs).[14–18] To combat infection, current clinical practice employs systemic antibiotics, which can be toxic, supplemented by local antibiotics in the form of non-biodegradable antibiotic-impregnated polymethymethacrylate (PMMA) beads, which will require removal at a later date.

This paper describes 3 years of research conducted by visiting British Military research fellows at the US Army Institute of Surgical Research (USAISR) exploring novel solutions to the twin problems of bone loss and infection following open fractures.

THE MODEL

Clinical studies in this area are challenging. Individual patients, their wounds and fractures are all different presenting multiple confounders to reliable outcomes, which can only be mitigated by studying large numbers of patients. This is commonly expensive, time consuming and logistically difficult. Animal models allow for standardisation, but careful selection of the model and methodologies are important to ensure they are relevant to the clinical problem under consideration.[19]

The basic extremity injury model used at USAISR is a critical-sized defect in the rat femur[16] with the use of a polyacetyl plate applied to the bone. The plate has notches 6 mm apart in the centre, to define where the bone defect is cut out, and three holes on either side, through which K wires are passed to fix the implant to the femur. This produces a standardised and reproducible bone defect which will not heal spontaneously as the bone ends are held apart by the polyacetyl plate; infection studies contaminate the defect with *S aureus*. The strength of this particular model is that it can be used for infection, bone healing and bone healing in an infected defect.

STUDIES ON THE TIMING OF TREATMENT

The current clinically accepted timeline for administration of systemic antibiotics for open fractures is 3 h,[20] though the clinical evidence to support this is weak. Furthermore, there is no evidence to support the use of local antibiotics alone in the treatment of infected fractures. To investigate the relevance of delays in treatment, all experimental animal groups had the critical-sized bone defect contaminated with 10^5 colony forming units of *S*

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aureus. Definitive treatment (irrigation and debridement followed by direct closure or implantation of antibiotic-impregnated PMMA beads) was delayed by 2, 6 or 24 h and subsequent bacterial load in the wounds quantified 2 weeks later. Bacterial counts in the bone increased significantly with the increasing delays to irrigation and debridement up to 24 h; the addition of local antibiotics significantly reduced the bacterial load up to 6 h, after which no difference was observed whether or not antibiotics were used (Table 1).[21]

To further mirror the clinical situation encountered in combat wounds where bacterial contamination persists, half of a group of animals were treated with the antibiotic cephazolin into the skin wound for 72 h beginning at the same time as surgical debridement 6 h after injury.[11] This provided a group of animals that still had bacteria in the bone defect 14 days after contamination. The effects of delaying the surgery and systemic antibiotics were also examined. Delaying antibiotic administration is more detrimental than delaying surgery with respect to the effect on subsequent bacteria load in the wound.[22]

These findings support the efforts of the DMS to reduce transit times between point of wounding and surgical management.

Wound irrigation

There has been little clinical or preclinical evidence demonstrating that irrigating wounds with antiseptic solutions is superior to saline at reducing infection in open fracture wounds, yet many surgeons still use antiseptics.[23] Chlorhexidine is an antiseptic that has a low toxicity to mammalian tissue despite exerting a potent antimicrobial effect. Comparison of various concentrations of chlorhexidine with saline to wash out a contaminated rat femur defect showed that chlorhexidine was not superior to saline at reducing bacterial loads in the wound 14 days after 'injury'.[24]

This is believed to be explained by the findings of Owens *et al*[25] that although antiseptics initially reduce the bacterial load in the wound, there is a subsequent 're-bound' of growth due to concurrent host tissue damage.

These findings support the current clinical practice of only irrigating combat wounds with saline and caution against the use of disinfectants for this purpose.

Delivery of local antibiotics

A collaboration with a pharmaceutical company (Dr Reddy's Laboratory Inc.) investigated a biodegradeable phospholipid gel as a delivery vehicle for gentamicin and vancomycin. This gel is designed to be placed in the wound prior to closure in order to deliver a local dose of antibiotics throughout the wound and does not require removal. It was selected for examination as it was believed there was an obvious potential for use in contaminated combat injuries. When tested in the contaminated rat femur defect model, this antibiotic gel was significantly superior to the current standard of PMMA beads at reducing detectible bacteria on both hardware and bone samples removed from animals 14 days after contamination and treatment, as shown in Figure 1.[26] Phase I clinical tests of this antibiotic gel are now planned.

Table 1 Bacteria concentration (colony forming units (CFUs)/g bone tissue) in each treatment group at the varying treatment time points

Time delay to treatment	Bacterial concentration (CFUs/g bone tissue)		
Time of	No antibiotics	Antibiotics	p Value
2 h	$1.98\times10^5\pm6.61\times10^4$	$3.00\times10^4\pm1.58\times10^4$	<0.01
6 h	$3.68\times10^6\pm9.23\times10^5$	$1.12\times10^6\pm3.41\times10^5$	0.016
24 h	$2.03\times10^7\pm7.56\times10^6$	$1.42\times10^7\pm4.13\times10^6$	0.46

The values are expressed as the mean±SE of the mean. Within the 'no antibiotics' group, there were differences between the 2 and 6 h groups but not the 6 and 24 h groups, whereas there were significant differences at all three time points in the antibiotics group (p<0.05). Reproduced with permission of the *Journal of Surgical Orthopaedic Advances*.[21]

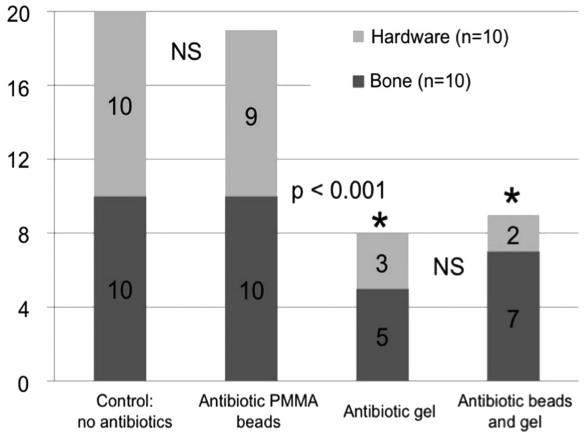

Figure 1 Proportion of 20 samples from each treatment group (10 animals per group) with detectible bacteria at 14 days. Statistical differences by Fisher's exact test are shown. *The antibiotic gel and antibiotic beads and gel group had a lower portion of the samples with bacteria that were recoverable than the other control and beads groups ($p\leq0.0004$). NS, no significant difference between adjacent groups.[26]

DUAL-DELIVERY SCAFFOLD

The ideal endpoint would be the development of a multifunctional scaffold with the dual capabilities to *eradicate* contaminating bacteria and *repair* large bone defects. Polyurethane (PUR) scaffolds could potentially satisfy the criteria to successfully deliver both growth factors such as BMPs[27] and antibiotics,[28] but this had not yet been described in a clinically relevant model. The concept was therefore evaluated in a series of iterative studies.

PUR with BMP

Using PUR scaffolds with rhBMP in the stringent critical-sized rat femur defect model, bone regeneration was compared with the current clinical standard, which is a collagen sponge soaked with rhBMP. There was 50% more bone regenerated in the defect by the PUR+BMP scaffold compared with the collagen sponge. This increase in bone formation is believed to be because of the more optimal release kinetics of the growth factors, which is a bolus release followed by a sustained release (Figure 2).[29]

PUR with vancomycin

To investigate if contamination could be controlled in the critical-sized defect using PUR scaffold with antibiotics in a comparable fashion with antibiotic-PMMA beads, the defects were irrigated, debrided and filled with the appropriate treatment implant 6 h after contamination. Both groups showed significant inhibitory effects on bacteria compared with the untreated control, and these two groups fared equally well.[30]

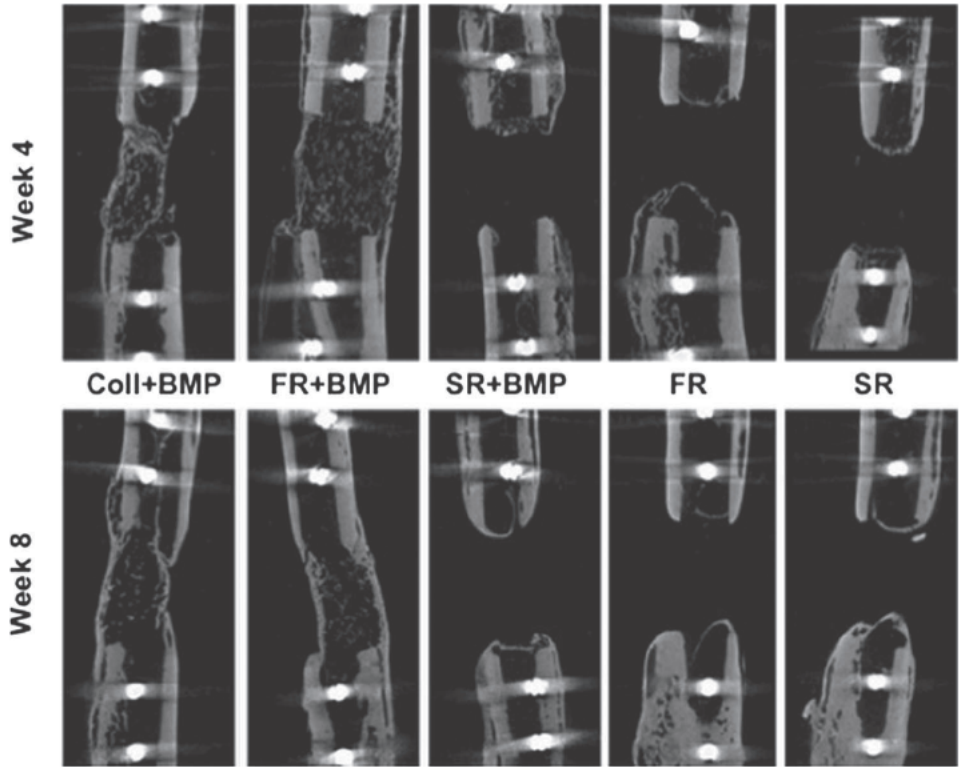

Figure 2 Representative microCT images of new bone formation in implants both with and without BMP (Coll, collagen implant; BMP, bone morphogenetic protein; SR, slow release polyurethane scaffold with no BMP; FR, fast release scaffold with no BMP; SR+BMP, slow release scaffold with BMP; FR+BMP, fast release scaffold with BMP).[29]

PUR with vancomycin and BMP (dual-purpose graft)

The results from the previous two studies were used to formulate a dual-purpose bone graft, which prevents the scaffold from contamination by releasing an antibiotic for 8 weeks and promotes bone formation by releasing BMP. This approach demonstrated a reduction in clinical signs of infection within the wound and a robust healing response.[31]

The use of a dual-delivery implant has not yet been translated into clinical practice although there are ongoing clinical trials.

SUMMARY

At USAISR, treatment concepts, strategies and novel treatments have been investigated over 3 years using animal models to test various concepts and their relevance to our clinical scenario to improve the outcomes of limb salvage. The choice of irrigation fluids and the comparison of timing of antibiotic and surgical treatment were tested which inform both surgeons and military commanders as to clinical timelines, prioritisation of treatments and resource planning.

Novel delivery of local antibiotics has consistently been shown in multiple studies over all 3 years to be superior to the current clinical standard of care. The concept of a dual-purpose bone graft was developed and evaluated, demonstrating that delivery of antibiotics along with a growth factor can be successful in healing contaminated bone defects.

The work performed during this 3-year collaboration between the UK Ministry of Defence and the USAISR has been used to create new clinical practice guidelines and help transition new therapies to the clinical arena.

Key messages

- Importance of short transit times.
- Irrigation should only be normal saline.
- Work continues on optimising local delivery of antibiotics.

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